OPTIMIZATION OF PROCESSING PARAMETERS FOR MINIMIZATION OF LIMONIN AND NARINGIN CONTENT IN KINNOW JUICE

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Abstract
The present study was carried out to evaluate the effect of three variables viz: naringinase, florisil and thermosonication on limonin and naringin content of kinnow juice. Box-Behenken design was used for design of experiment. Total 17 experiments were suggested by Design expert trial version (13.0) software, out of these 17 experiments there were 5 center point experiments. Each variable was varied at (-1), (0) and (+1) levels. Florisil and naringinase content was varied from 20g/L to 40g/L and 0.6mL/100mL to 1.0 mL/100mL while thermosonication of juice samples was performed from low (20KHz) to high frequency (40 KHz) at 30^0C for 20 minutes in a portable thermosonicator machine. For each experiment 200mL kinnow juice was taken in 500mL glass beaker. Initially florisil treatment was given (2 minutes stirring followed by 2 minutes rest to ensure sedimentation of adsorbent). In the next step thermosonication (Power sonic 410) of juice sample was carried out for 20 minutes at 30^0C at different frequencies by transferring the juice in falcon tube (45 mL) and for each sample 03 tubes were taken. In the end naringinase treatment was given by transferring the calculated amount of enzyme in juice sample (50 mL) and incubating it for 12 hours at room temperature. Limonin and naringin content of treated samples, fresh juice and different parts of kinnow fruits were estimated, there values were as follows: fresh juice (7.8 ppm and 212.0 ppm), peel (46.0 ppm and 13121.0 ppm), pomace (190.0 ppm and 150.0 ppm) and albedo (negligible and 3928.0 ppm). Reduction in limonin and naringin content was found varying from 45.6 to 51.2% and 33.3 to 40.9%, respectively. The least deviation in actual values (limonin - 43.8% reduction and naringin - 40.0 % reduction) of response against predicted values (limonin - 45.64% reduction and naringin - 40.3 % reduction) was found in condition at naringinase (1.0 mL/100 mL), florisil (20 g/L) and thermosonication treatment (37.21 KHz).

Keywords: Limonin, Naringin, kinnow juice

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INTRODUCTION

Citrus fruits are India's third most popular fruit with an estimated production 13976000MT and area under cultivation 1054000 hectares after bananas (31504000 MT) and mangoes (20444000 MT), as per a recent report released by National Horticulture for 2nd advance estimate of year 2019-2020. For the year 2018-2019 citrus fruits production in Haryana was estimated 549332 MT while the total area under cultivation was 20789 hectares. District Sirsa was ranked first with production of 306724 MT followed by Fatehabad (70515 MT), Bhiwani (55226 MT), Narnaul (39687 MT) and Hisar (28417 MT) (Horticulture Department of Haryana, 2018-19) for production of citrus fruit. Mosambi, kinnow, orange, lemon, lime, galgal, tangerine and grapefruit are common citrus fruits grown in India (Purewal *et al*., 2021).

Kinnow (*Citrus reticulata* Blanco.) is a citrus fruit from family *Rutaceae.* It is a versatile fruit and grows well at altitudes of 500-1500 MSL, 150-250 cm rainfall, in subtropical climate with moderate winters and warm summers. Under adequate management the tree bears the fruit from third year and continues so for 15-20 years. Kinnow at the maturity time of mid-January to mid-February has a TSS/acid ratio of 12:1 - 14:1 and a golden orange colour while in the initial periods of growth it is green in colour. Kinnow peel is categorized into two sections: exterior brightly colored layer known as flavedo which contains bright pigments and oil cells, which is used as a raw material to extract essential limonene oil while albedo is the inner white colour fiber layer, which is high in

pectin and is bitter due to presence of naringin. Seeds are found in the middle of the fruit axis in numbers ranging from 5 to 23 which vary according to the size, variety, and maturity of the fruit. The seeds are bitter and contain limonin. Expressing, hydraulic pressing and squeezing can all be used to extract juice from citrus fruits (peeled or intact). The juice obtained by peeling, squeezing fruit and soft press extraction in such a manner that seeds are not crushed was shown to be superior to other techniques, with the sole drawback being a lower juice recovery (Premi *et al*., 1994). The average vitamin C content of kinnow is 31.0mg/100g, iron 0.4mg/100g, calcium 40mg/100g, average TSS% 11.5, phosphorus 18mg/100g and average acidity is 0.9 % (Rattanpal *et al*., 2017). Currently 95% of kinnow output is destined for the fresh fruit market and during seasonal peaks, there is a surplus in the market, prices decrease and kinnow is wasted (25-30%) (Khandelwal *et al*., 2006). The total post-harvest waste from farms to Kinnow customers ranged from 14.87 percent in the Delhi market to 21.91 percent in Bengaluru market (Gangwar *et al*., 2007). Processing of kinnow juice into value added product can be possible solution to these postharvest losses. Apart from highly perishable and lesser shelf-life citrus juices also possesses another problem of bitterness and this problem is difficult to overcome for any citrus industry. Excessive bitterness in citrus juice is a big issue in the citrus business across the world since it lowers the product's quality and economic value (Mongkolkul *et al*., 2006).

Limonin and naringin are two key components, responsible for bitterness in kinnow juice. Limonin is a type of limonoid, which is mainly present in seeds of fruit while naringin is a type of flavonoid which is present in skin and juice of the kinnow fruit. The concentration of limonin and naringin in citrus fruits may vary with the type of fruit, cultivar, agroclimatic condition where it is grown. It also varies in different parts of fruit (peel, seeds, albedo etc). Limonin is generated in leaves as a non-bitter form (limonoate A ring lactone) and is transferred to fruit and seeds. As a result, the amount of limonin in each section of the fruit varies (Maier *et al*., 1977). The entire fruit contains almost no limonin but its non-bitter precursor which is limonate-A-ring lactone (LARL) is shown to be intracellularly present in the cytoplasm of cells in membranous sacs, most likely at a neutral to slightly alkaline pH. When these sacs burst during juice extraction, the LARL comes in contact with the juice's total acidic pH which catalyses ring closure to form limonin (Kimball, 1991). The presence of an endogenous enzyme limonin D-ring lactone hydrolase accelerates this conversion by catalysing D-ring lactoneization under acidic circumstances or opening the o-ring of limonin under alkaline circumstances (Maier *et al*., 1969). Kumar *et al*., 2020 reported that limonin content kinnow fruits varied in different parts, seeds (224.37 ppm), pulp (114.91 ppm), flavedo (56.95 ppm) and juice (20.33 ppm) whereas negligible amount was found to be in the albedo portion. Kaur *et al*., 2018 reported limonin content of fresh kinnow juice to be 7.50 ppm. Naringin is a water-soluble bitterness causing compound present in the fruit membrane and albedo and is extracted into fruit juices (Fisher and Wheaton, 1976). Peel has maximum naringin content followed by seeds of citrus fruits, that's why citrus fruits are scraped before extraction of juice (Garcia and Castillo, 2008). It is most bitter flavonoids amongst naringin, poncirin, neohesperidin, and neoeriocitrin (Hasegawa *et al*., 1996). Kumar *et al*., 2020 reported the naringin content in different parts of kinnow fruit and found that the highest concentration was in flavedo (13589.82 ppm), albedo (4037.83 ppm) seed (710.82 ppm), pulp (131.84 ppm) and the least was found to be in juice (105.67ppm).

Removal of bitterness causing compounds from juice, removal of physical barriers, use of flavor enhancers and bitter compounds scavengers (salt, sugar, florisil), enzymatic (naringinase and α-L-rhamnosidase) treatment, genetic engineering techniques are some basic approaches used to minimize bitterness. Researchers have already used lye treatment, addition of sugars, β-cyclodextrin, hot water treatment, cellulose acetate layers, enzymatic

methods using microbial consortia as a solution of this problem. Attempts have already been made to reduce these two principlecomponents in the citrus juices but it has not been eliminated totally also the treatments given changed the sensory and nutritional quality of the juice. The present study focused on the combination of techniques to reduce the bitterness. Response surface methodology was used for design of experiments. Three variables viz: crude naringinase, florisil and thermosonication were used in Box Behenken Design. The present study was carried out to evaluate the the effect of Naringinase enzyme (crude form), thermosonication and florisil conent in juice to minimize the concentration of limonin and naringin. Florisil is an odorless, white colour adsorbent which is used as a debittering agent, chemically it is activated magnesium silicate (Purewal *et al.* 2020). Treatment of kinnow and other citrus juices with florisil may resulted in reduction in the limonin content and naringin content (Kumar *et al*., 2020, Barmore *et al*., 1986, Nikdel *et al*., 1987, Chaisawadi *et al*., 1998). Thermosonication is a treatment that combines heat and sonication in the product is treated with moderate heat (Aadil *et al*., 2015), it has minimum effects on juice quality (Tiwari *et al*., 2009). Xianli *et al*., 2021 found that due sonication the degrees of enzymatic hydrolysis of limonin and naringin were increased to 36.16 percent and 89.90% respectively, while the enzymatic hydrolysis duration was reduced by 33%. It was also reported that sonication increased the activity of enzymes also helps in breaking of C O bonds in naringin. Naringinase is a multifunctional enzyme that utilizes naringin as a substrate and converts it to rhamnose and prunin using its alpha-L-rhamnosidase activity subsequently beta-D-glucosidase breaks down prunin into glucose and naringenin. The quantity of bitter naringin is reduced by producing naringenin which has a sweet flavour therefore the enzyme is commonly employed in the debittering of citrus juice (Narnoliya *et al*., 2019, Puri *et al*., 2005, Silva *et al.,* 2017, Kaur *et al*., 2018). The enzyme alpha-L-rhamnosidase hydrolysis naringin to prunin (33 percent as bitter as naringin) and L-rhamnose as the fruit grows and the least amount of naringin is found in ripened fruit. Prunin is further acted upon by beta-D-glucosidase which converts it to naringenin and D-glucose. Thus, naringin hydrolysis mediated by alpha- L rhamnosidase and beta-D-glucosidase generates substantially debittered, consumer acceptable citrus juice (Puri, 1993).

MATERIAL AND METHODS

The present study was carried out in the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar.

Materials:

Kinnow fruit: Fresh kinnow fruits were procured from the local market of Hisar, Haryana.
Naringinase: Naringinase enzyme used in study was prepared by using *Aspergillus niger* strain. *Aspergillus niger* was grown in 250 mL conical flask having 150 mL sterilized nutrient broth mixed with naringin (1 gm). Flask was kept in shaking incubator at 25-27 ^{0}C followed by centrifugation (5000 rpm for 10 minutes). Supernatant thus collected was used in the present study as crude form of the enzyme.
Florisil and other chemicals: Florisil used in study was supplied by central drug house (CDH) private limited. Other chemicals used in this study were also laboratory grade and supplied by reputed agencies.

Methods:

Extraction of Kinnow juice
Fresh and healthy fruits free from blemish and any kind of spots were taken for juice extraction. Fruits were washed and dried before slicing them into two halves with the help of a knife. Two halves thus obtained were placed on a cone (perforated) of a potable juice extracting machine. The fruit sacs were compressed between fixed cone and an upper moving arm, manually. Compression leads to extraction of juice which was collected in laboratory glass beaker (500 mL), later it was transferred to bottles after removal of fibrous part by straining through a sieve. This technique doesn't not

crush the seeds and also does not allow the pulp to mix in the juice, which was required for present research work. Incorporation of pulp and crushing of seed may add on bitterness to juice.

Preparation of peel, pomace and albedo powder

Prior to collection of fruit peel manually, fruits were washed thoroughly to remove any adherent impurities. The albedo portion of the fruit was also removed manually. Pomace used in study was collected from juicer machine after extraction of juice (albedo free fruit). Peel, pomace and albedo were subjected to cutting into smaller pieces with help of kitchen knife. Freeze drying was carried out by placing the smaller pieces of each type of sample in petri plates to access the actual content of naringin and limonin. Samples were freezed for 3 hours at - 2^0C and then were freeze dried for 10 hours. Samples collected after drying were grounded using kitchen grinder (Sujata).

Processing of kinnow juice

To minimize the concentration of bitterness causing compounds, A Box- Behenken design was used. Three variables namely: naringinase enzyme, thermosonication and florisil were selected to decrease the limonin and naringin content of freshly extracted kinnow juice. The level of these variable was selected on the basis of preliminary studies and literature reviewed. Florisil and naringinase content was varied from 20g/L to 40g/L and 0.6mL/100mL to 1.0 mL/100mL, respectively (**Table 1**). Thermosonication of juice samples was performed from low (20KHz) to high frequency (40 KHz) at 30^0C for 20 minutes in a portable thermosonicator machine. Each variable was varied at (-1), (0) and (+1) levels. Total 17 experiments were suggested by Design expert trial version (6.0) software, out of these 17 experiments there were 5 center point experiments. For each experiment 200mL kinnow juice was taken in 500mL glass beaker. Initially florisil treatment was given by transferring the weighed amount of florisil calculated according to design of experiment, followed by stirring at 2 minute using high speed laboratory stirrer. After stirring samples were allowed to rest for 2 minutes for sedimentation of adsorbent and then straining of juice was carried out by using double muslin cloth. In the next step thermosonication (Power sonic 410) of juice sample treated with florisil, was carried out for 20 minutes at 30^0C at different frequencies by transferring the juice in falcon tube (45 mL) and for each sample 03 tubes were taken (**Fig. 1**). In the end naringinase treatment was given by transferring the calculated amount of enzyme in juice sample (50 mL) and incubating it for 12 hours at room temperature. After treating the samples with all the three techniques the samples were analyzed for limonin and naringin content.

Estimation of limonin content (Abbasi *et* al., 2005)

Limonin content of kinnow juice was determined using spectrophotometric method. Reagents used were Acetonitrile, chloroform, glacial acetic acid, perchloric acid (70%), 4(dimethylamino)benzaldehyde and D limonin. Burnham's reagent was prepared by - 0.1 g DMAB, 3mL acetic acid, 2.4 ml perchloric acid. Standard stock solution of limonin (10ppm): 0.5 ml limonin was taken in 50 mL volumetric flask and volume was made up 50 ml by using acetonitrile. For preparation of standard curve 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken and the volume was made upto 1 ml by distilled water and further following procedure was followed. The juice samples were diluted two times and then centrifuged for 10 minutes at 3,000 rpm and the supernatant was taken for further estimation and in the case of freeze dried samples 1 gram of sample was weighed and diluted 100 times with distilled water, it was then centrifuged at 4000 rpm/5 minutes and was filtered using whattman paper no. 4. In order to eliminate polar substances, 1 ml of the supernatant was placed into a test tube, succeeded by 2 ml chloroform. A shaker was used to thoroughly mix the mixture for 2 minutes which leaded to phase separation, 1.5 ml of Burnham reagent (0.1 g 4-(dimethylamino) benzaldehyde, glacial acetic acid (3 ml), and perchloric acid (2.4 ml) was added to 1 ml of the chloroform phase.To get the most reddish colour, this combination was left at room temperature for 30 minutes. At 503nm, the top

Table: 1. Values of independent variables at three levels of the Box-Behnken design

Independent variables	Code	Levels in coded form		
		-1	0	+1
Naringinase (mL/100mL)	x_1	0.6	0.8	1.0
Florisil (g/L)	x_2	20	30	40
Thermosonication (KHz)	x_3	20	30	40

phase's absorbance was observed. After noting down the absorbance the concentration of the samples was calculated from the standard curve graph (absorbance vs concentration) in ppm.

Estimation of naringin content (Prakash *et al*., 2002)

Naringin content was estimated by Davis method (1947). Reagents required - standard naringin, 90% diethylene gylycol, 4M sodium hydroxide (160 gram in 1000 ml distilled water), standard stock solution (200 ppm) - 10 mg of standard naringin was weighed and the volume was made upto 50 ml with distilled water.

Procedure: Standard curve - 0.2, 0.4, 0.6, 0.8 and 1 ml of the stock solution was added in separate test tubes and the volume was made upto 1 ml with distilled water followed by 10 ml of 90% diethylene glycol and 0.2 ml of 4M sodium hydroxide. The test tube was incubated for 5 minutes at room temperature and the readings were noted using UV spectrophotometer at 420 nm .The juice samples were diluted 10 times and in the case of freeze dried samples 1 gram of sample was weighed and diluted 100 times with distilled water, it was then centrifuged at 4000rpm / 5 minutes and was filtered using whattman paper no. 4. The readings were noted down for the absorbance of each sample. The concentrations were calculated from the standard curve graph (absorbance vs concentration).

Minimization of limonin and naringin content (%): For calculation of % degradation in both compounds, following formula was used

% Minimization
= Initial content (Fresh juice) - Final content (processes juice) / Initial content (Fresh juice) *100

Data analysis and modeling

A Box-Behnken experimental design (Box and Behnken, 1960) was used to study the effect of independent variables on dependent variables. The independent variables level was selected through preliminary trials and feasibility of operating condition. The experimental design involved 17 experiments with 5 combinations of the central point and presented in **table 2.** Design Expert trial version 13 (State-Ease, Minneapolis, MN) was used for analysis of variables. The response (limonin and naringin content) for different experimental combinations was related to the coded variables (xi, i=1, 2 and 3) by a second-degree polynomial equation

$$Y=\beta 0+\beta 1x1+\beta 2x2+\beta 3x3+\beta 12x1.x2+\beta 13x1.x3+\beta 23x2.x3+\beta 11x12+\beta 22x22+\beta 33x32$$

Where Y is the estimated response, the coefficients of the polynomial were represented by $\beta 0$ (constant), $\beta 1$, $\beta 2$, $\beta 3$ (linear effects); $\beta 12$, $\beta 13$, $\beta 23$ (interaction effects); $\beta 11$, $\beta 22$, $\beta 33$ (quadratic effects). The adequacy of the model was determined by evaluating the lack of fit, coefficient of correlation (R2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA). The regression coefficient was used to generate response surface three-dimensional plots by keeping one response at the centre level.

Fig. 1. a) Fresh kinnow fruit, b) Removing albedo and flawed parts, c) Juice extraction, d) Florisil treatment, e) Filtering with double layer of muslin cloth, f) Thermosonication treatment, g) Enzyme treatment

Table: 2. Limonin and naringin content different parts of kinnow fruit and juice

Sr. No.	Part of fruit	Naringin (ppm)	Limonin (ppm)
1	Fresh juice	212.0	7.8
2	Peel (flavedo)	13121.0	46.0
3	Pomace	150.0	190.0
4	Albedo	3928.0	Negligible

RESULTS AND DISCUSSIONS

Limonin and naringin content different parts of kinnow fruit and juice

Limonin and naringin content in different parts of kinnow fruits were as follows, respectively: fresh juice (7.8 ppm and 212.0 ppm), peel (46.0 ppm and 13121.0 ppm), pomace (190.0 ppm and 150.0 ppm) and albedo (negligible and 3928.0 ppm) as shown in **table 2**. Limonin content was highest in the pomace while naringin was highest in the albedo, similar results were also reported by Kumar *et al*., (2020) and Kaur *et al*., (2018). Results had indicated that if the peel, pomace and albedo parts are removed carefully from the fruit then the amount of limonin and naringin in the juice can be reduced to a significant amount.

Effect of naringinase, florisil and thermosonication treatments on minimization of naringin and limonin content of kinnow juice

Reduction in limonin and naringin content was found varying from 45.6 to 51.2% and 33.3 to 40.9%, respectively (**Table 3**). F value 129.64 implies that model term was significant for naringin content of juice (**ANOVA Table 4**). Only linear effect of variables was found significant, whereas interactive effect and quadratic effect was not observed. R square value 0.9676 and adj-R square value was also closer to 1. The cube plot (**Fig. 2**) was used to illustrate the combined effect of three variables on naringin percentage reduction of kinnow juice. The values indicated in the plots are predicted by software after analysis of variance. It was realized that the maximum reduction naringin percent can be obtained at highest level of all selected variables. While the minimum reduction in naringin percent would be at minimum level of variables. From the cube plot for naringin it was predicated that the maximum reduction in naringin (41.50%) was at (+1) level of all selected variables i.e., 1ml/100mL Naringinase, 40g/L florisil and 40 KHz Thermosonication frequency while minimum reduction in naringin content of kinnow juice was expected at (-1) level of all three variables. Highest reduction in naringin percent of kinnow juice was followed at combination of (+1) level of A and B, (-1) and (-1) level of C. With increasing concentration of enzyme naringinase, the percentage reduction in naringin was found increasing .It was found that with increasing concentrations of enzyme naringinase(from 0.6ml/100ml to 1 ml/100ml) there was a linear increase in the amount of percent naringin reduction, because naringinase is a multifunctional enzyme that performs two functions: alpha-L-rhamnosidase and beta-D-glucosidase , takes naringin as a substrate and converts it to rhamnose and prunin using its alpha-L-rhamnosidase activity; while beta-D-glucosidase activity breaks down the prunin into glucose and naringenin. As it lowers the quantity of bitter naringin and produces naringenin (sweet flavour) it decreases the amount of bitter principle naringin in the juice. Puri *et al*., 2005 treated kinnow mandarin juice with immobilised naringinase, and found that 76 percent of naringin was hydrolyzed in 1 hour. Silva *et al.,* 2017 observed that best reduction was achieved by adding 1.0g/L naringinase enzyme at 50°C for 4 hours with an 86 percent decrease in bibla sweet oranges. Similar results were also reported by Housseiny and Aboelmagd, 2019, Kaur *et al*., 2018 and Patil and Dhake, 2014.

Table: 3. The Box–Behnken design matrix used for treatment and the responses of kinnow juice

Sr. No.	Naringinase (mL/100mL)	Florisil (g/L)	Thermosonication (KHz)	Naringin (% reduction)	Limonin (% reduction)
1	0.6	20	30	33.3	45.9
2	1	20	30	40.9	45.6
3	0.6	40	30	34.4	49.7
4	1	40	30	41.5	49.0
5	0.6	30	20	34.0	48.6
6	1	30	20	39.6	46.0
7	0.6	30	40	34.7	49.0
8	1	30	40	40.0	48.2
9	0.8	20	20	36.0	45.8
10	0.8	40	20	37.0	49.5
11	0.8	20	40	37.1	46.2
12	0.8	40	40	38.6	51.2
13	0.8	30	30	37.4	48.5
14	0.8	30	30	37.4	48.5
15	0.8	30	30	37.4	48.5
16	0.8	30	30	37.4	48.5
17	0.8	30	30	37.4	48.5

It was also seen that with increasing concentrations of florisil the percent reduction of naringin of the juice increased linearly. Kumar *et al*., 2020 used florisil on kinnow juice and observed that it reduced the limonin content to 50 %. Florisil decreased the amounts of both naringin and limonin in the kinnow juice. Barmore *et al*., 1986 reported a decrease in the naringin content of grapefruit juice when florisil was used.

Table: 4. Analysis of variance for naringin content of kinnow juice

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	85.93	3	28.64333	129.7433	< 0.0001	significant
x_1	81.92	1	81.92	371.0662	< 0.0001	
x_2	2.205	1	2.205	9.987805	0.0075	
x_3	1.805	1	1.805	8.175958	0.0134	
R-Squared	0.96768					
Adj R-Squared	0.960222					
Pred R-Squared	0.932889					

Level of significance: *P<0.1, **P<0.05, ***P<0.01, df: degree of freedom

It was also observed that with the increasing frequencies of sonication treatment the percent reduction in naringin was found to increase in the juice and a possible reason for this decrease was reported by Xianli et al., 2021, sonication increased the activity of -l -rhamnosidases, -limoninases and glucosidases and it also helps in breaking of C O bonds in naringin.

Relationship established between dependent and independent variable for naringin content of juice was predicted as follows:

Naringin (%) = +37.30 + 3.20 * x_1 + 0.53* x_2 + 0.48* x_3

F value for given model is 30.25 (**ANOVA Table 5**) the value of "Prob. > F" for model is also less than 0.05, had indicated that the model was significant, which is desired. Linear, quadractic and interactive effect found for following model terms, x1, x2, x3, x12 and x13. The value or R squared should be between 0-1 and in this case, it is high (0.9749) and close to 1, which is also desired. **Fig. 3** revealed that maximum reduction in limonin (50.68%) was found at highest level (40 g/L and 40 KHz) of B and C variables, respectively by keeping Naringinase content at 0.6 mL/100 mL level. With increasing concentration adsorbent florisil, limonin percent of the juice was found to be increasing. It was observed that with the increasing concentration of enzyme naringinase the amount of percent reduction in limonin decreased a little and it could possibly be due to the long incubation time. The application of adsorbent florisil resulted in a sharp increase in the percent reduction of limonin of the juice, attributed to the adsorbing power of florisil. Results were in accordance with finding of Kumar *et al*., 2020. Barmore *et al*., 1986 reported that florisil (activated magnesium silicate) treatment of commercial grapefruit juice decreased the amount of citric acid as well as the bitter chemicals limonin and naringin. Nikdel *et al.,* 1987 treated single-strength grapefruit juice with three types of Florisil (B-Florisil, K-Florisil and C-Florisil) and found out that flavanone glycosides and limonin content were reduced by all three kinds of Florisil. Similar findings were reported by Chaisawadi *et al*., 1998. Valero *et al*., 2007 worked on orange juice and found out that in both static and continuous circumstances, limonin production appeared to be independent of treatment, with the exception of ultrasonic treatments mixing low and high frequencies. It was only when additional pulp was added to the juice that limonin content was slightly increased. Similarly in the present study there was no pulp of fibrous part in the juice and therefore a slight decrease was seen in the limonin content.

Table: 5. Analysis of variance for limonin content of kinnow juice

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	40.16279	9	4.462533	30.25446	< 0.0001	significant
x_1	2.42	1	2.42	16.40678	0.0049	
x_2	31.60125	1	31.60125	214.2458	< 0.0001	
x_3	2.76125	1	2.76125	18.72034	0.0035	
x_1^2	1.453289	1	1.453289	9.85281	0.0164	
x_2^2	0.553289	1	0.553289	3.751115	0.0940	
x_3^2	0.005921	1	0.005921	0.040143	0.8469	
x_{12}	0.04	1	0.04	0.271186	0.6186	
x_{13}	0.81	1	0.81	5.491525	0.0516	
x_{23}	0.4225	1	0.4225	2.864407	0.1344	
R-Squared	0.9749					
Adj R-Squared	0.9427					
Pred R-Squared	0.5990					

Level of significance: *P<0.1, **P<0.05, ***P<0.01, DF: degree of freedom

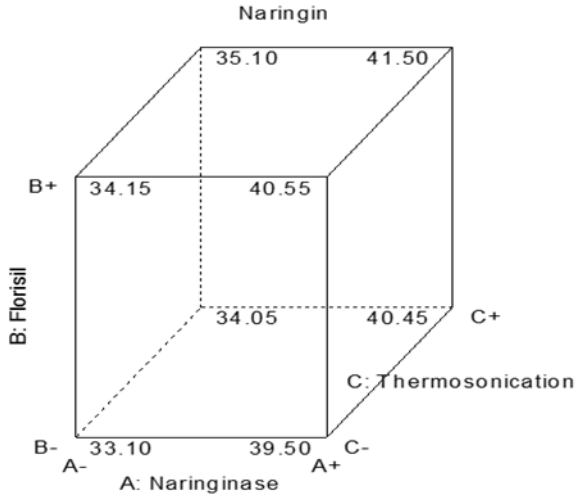

Fig. 2. Cube plot for Effect of naringinase, florisil and thermosonication treatment on minimization naringin content of kinnow juice

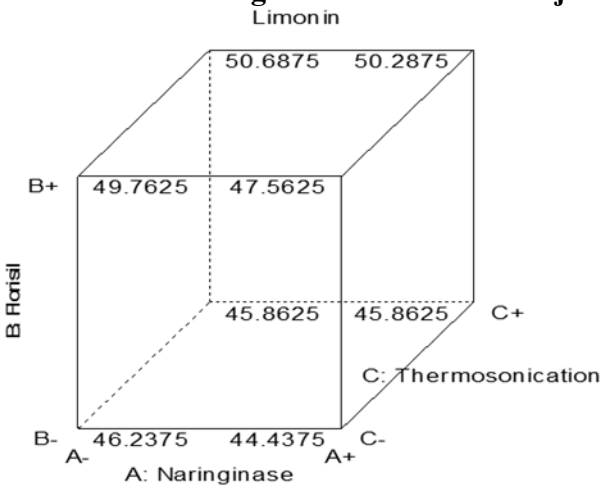

Fig. 3. Cube plot for Effect of naringinase, florisil and thermosonication treatment on minimization limonin content of kinnow juice

Model predicted for limonin content of juice, was as follows:

$$\textbf{Limonin} = +48.50 - 0.55 x_1 + 1.99 x_2 + 0.59 x_3 - 0.59 x_1^2 - 0.36 x_2^2 + 0.038 x_3^2 - 0.10 x_{12} + 0.45 x_{13} + 0.32 x_{23}$$

Optimization

The numerical multi-response optimization technique with desirability function was used to estimate the optimum level of naringinase, florisil and thermosonication. The least deviation in actual values (limonin - 43.8% reduction and naringin - 40.0 % reduction) of response against predicted values (limonin - 45.64% reduction and naringin - 40.3 % reduction) was found in condition at naringinase (1.0 mL/100 mL), florisil (20 g/L) and thermosonication treatment (37.21 KHz) (**Table 6**). Therefore, it was found best among respective 3 optimum conditions having less than 5% deviation. Hence the optimized conditions were considered further to study.

Table: 6. Optimized level of variables and responses

	Naringinase (mL/100mL)	Florisil (g/L)	Thermosonication (KHz)	Naringin (% reduction)	Limonin (% reduction)
Predicted	1.00	20.00	37.12	40.3111	45.64
Actual	1.00	20.00	30.0	40.0	43.8

CONCLUSION

Bitterness causing compound were minimized by the selected combination of variables, although they were present comparatively in lower amount because fruits were procured in month of March. Reduction in limonin and naringin content was found varying from 45.6 to 51.2% and 33.3 to 40.9%, respectively. Naringin content kinnow juice was found decreasing with increasing level of all three variable; of naringinase, florisil and thermosonication. Impact of naringinase enzyme was more profound for minimization naringin content. For limonin degradation florisil has shown a significant effect. Every year production of kinnow fruit is increasing in Sirsa, Fatehabad and Bhiwani district, outcome of this research can benefit both farmers and processer in future.

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